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(54) Title: METHODS OF AGONIZING AND ANTAGONIZING FABK

(57) Abstract: The invention provides methods for using agonists and antagonists of FabK polypeptides, particularly to modulate the metabolism of bacteria or to treat bacterial infection.

## Methods of Agonizing and Antagonizing FabK

### FIELD OF THE INVENTION

This invention relates to methods of agonizing or antagonizing polynucleotides and polypeptides of the Fab family, as well as their variants, herein referred to as "FabK," "FabK polynucleotide(s)," and "FabK polypeptide(s)," as the case may be.

### BACKGROUND OF THE INVENTION

Infections caused by or related to bacteria are a major cause of human illness worldwide, and the frequency of resistance to standard antibiotics has risen dramatically over the last decade. Hence, there exists an unmet medical need and demand for new anti-microbial agents against pathogenic bacteria, as well as drug screening methods to identify such agents.

An example of a bacterial enzyme that is resistant to a widely used antibacterial agent, Triclosan, is FabK. This enzyme, involved in fatty acid biosynthesis, has been recently reported from *Streptococcus pneumoniae*, a well-known human pathogen (Heath, *et al. Nature* 406: 145 (2000)). The specific activity of the enzyme under the published conditions was 64 +/- 4 nmol min<sup>-1</sup>, too low to efficiently screen for compounds that modulate the activity of the enzyme, such as inhibitors (Heath, *et al. Nature* 406: 145 (2000)). The present invention solves this problem by providing a method for screening for FabK agonists and antagonists, wherein FabK activity is sufficient to perform efficient compound screening.

A further problem identified by recent studies has been solved by this invention. Heath teaches that organisms expressing FabK will be refractory to FabI inhibitors, and that bacteria possessing both targets will require a combination of inhibitors to block growth (Heath, *et al. Nature* 406: 145 (2000)). The present invention provides methods of screening for compounds that inhibit both enzymes, as well as the use of such compounds as antimicrobial compounds.

### SUMMARY OF THE INVENTION

The present invention provides, among other things, methods are also provided for the treatment of bacterial infection comprising the step of contacting an individual with an antagonist or agonist of a FabK polypeptide.

The invention also provides a method for modulating the metabolism of bacteria comprising the step of contacting said bacteria with an antagonist or agonist of a FabK polypeptide.

Methods are also provided wherein said FabK polypeptide selected from the group consisting of: (i) an isolated polypeptide comprising an amino acid having at least 95% identity

to the amino acid sequence of SEQ ID NO:2 over the entire length of SEQ ID NO:2;

- 5 (ii) an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2,  
 (iii) an isolated polypeptide that is the amino acid sequence of SEQ ID NO:2, and  
 (iv) a polypeptide that is encoded by a recombinant polynucleotide comprising the polynucleotide sequence of SEQ ID NO:1.

Further methods are provided wherein said antagonist is a small molecule.

- 10 Still further methods are provided wherein said small molecule has the structure or Formula I, or a homologue, stereoisomer, or a pharmaceutically acceptable derivative thereof.

- A method wherein said small molecule has the structure or Formula II, or a homologue, or stereoisomer, or pharmaceutically acceptable derivative thereof is also  
 15 provided by the invention.

- A method is also provided wherein said bacteria is or said infection is caused by or associated with a bacteria selected from the group consisting of: a member of the genus *Streptococcus*, *Staphylococcus*, *Bordetella*, *Corynebacterium*, *Mycobacterium*, *Neisseria*, *Haemophilus*, *Actinomycetes*, *Streptomyces*, *Nocardia*, *Enterobacter*, *Yersinia*, *Fancisella*,  
 20 *Pasturella*, *Moraxella*, *Acinetobacter*, *Erysipelothrix*, *Branhamella*, *Actinobacillus*, *Streptobacillus*, *Listeria*, *Calymmatobacterium*, *Brucella*, *Bacillus*, *Clostridium*, *Treponema*, *Escherichia*, *Salmonella*, *Klebsiella*, *Vibrio*, *Proteus*, *Erwinia*, *Borrelia*, *Leptospira*, *Spirillum*, *Campylobacter*, *Shigella*, *Legionella*, *Pseudomonas*, *Aeromonas*, *Rickettsia*, *Chlamydia*, *Borrelia* and *Mycoplasma*, and further including, but not limited to, a member of the species or  
 25 group, Group A *Streptococcus*, Group B *Streptococcus*, Group C *Streptococcus*, Group D *Streptococcus*, Group G *Streptococcus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus faecalis*, *Streptococcus faecium*, *Streptococcus durans*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium diphtheriae*, *Gardnerella vaginalis*, *Mycobacterium tuberculosis*,  
 30 *Mycobacterium bovis*, *Mycobacterium ulcerans*, *Mycobacterium leprae*, *Actinomycetes israelii*, *Listeria monocytogenes*, *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica*, *Escherichia coli*, *Shigella dysenteriae*, *Haemophilus influenzae*, *Haemophilus aegyptius*, *Haemophilus parainfluenzae*, *Haemophilus ducreyi*, *Bordetella*, *Salmonella typhi*, *Citrobacter freundii*, *Proteus mirabilis*, *Proteus vulgaris*, *Yersinia pestis*, *Klebsiella*

*pneumoniae*, *Serratia marcescens*, *Serratia liquefaciens*, *Vibrio cholera*, *Shigella dysenterii*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Franscisella tularensis*, *Brucella abortis*, *Bacillus anthracis*, *Bacillus cereus*, *Clostridium perfringens*, *Clostridium tetani*, *Clostridium botulinum*, *Treponema pallidum*, *Rickettsia rickettsii*, *Helicobacter pylori* and *Chlamydia trachomatis*.

5 Also provided by the invention is a method using an antagonist or agonist of a FabK polypeptide that is also an agonist or antagonist of FabI.

A preferred method is provided wherein said modulating metabolism is inhibiting growth of said bacteria or killing said bacteria.

10 Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following descriptions and from reading the other parts of the present disclosure.

#### DESCRIPTION OF THE INVENTION

15 The invention relates to methods using FabK polypeptides and polynucleotides as described in greater detail below. In particular, the invention relates to methods using polypeptides and polynucleotides of a FabK of *Streptococcus pneumoniae*. The invention relates especially to methods using FabK having a nucleotide and amino acid sequences set out in Table 1 as SEQ ID NO:1 and SEQ ID NO:2 respectively. The start codon in SEQ ID NO: 1  
20 begins with nucleotide 1 and the stop codon ends with nucleotide 975. Note that sequences recited in the Sequence Listing below as "DNA" represent an exemplification of the invention, since those of ordinary skill will recognize that such sequences can be usefully employed in polynucleotides in general, including ribopolynucleotides.

25

TABLE 1

#### FabK Polynucleotide and Polypeptide Sequences

(A) *Streptococcus pneumoniae* FabK polynucleotide sequence [SEQ ID NO:1].

30

5'-

ATGAAAACGCGTATTACAGAATTATTGAAGATTGACTATCCTATTTTCCAAGGAGGGATGGCCTGGGT  
TGCTGATGGTGA  
TTTGGCAGGGGCTGTTTCCAAGGCTGGAGGATTAGGAATTATCGGTGGGGGAAATGCCCCGAAAGAAG  
TTGTCAAGGCCA

ATATTGATAAAATCAAATCATTGACTGATAAACCCCTTTGGGGTCAACATCATGCTCTTATCTCCCTTT  
 GTGGAAGATATC  
 GTGGATCTCGTTATTGAAGAAGGTGTTAAAGTTGTCACAACAGGAGCAGGAAATCCAAGCAAGTATAT  
 GGAACGTTTCCA  
 5 TGAAGCTGGGATAATCGTTATTTCCTGTTGTTCCCTAGTGTGCTTTAGCTAAACGCATGGAAAAATCG  
 GTGCAGACGCTG  
 TTATTGCAGAAGGAATGGAAGCTGGGGGGCATATCGGTAAATTAACAACCATGACCTTGGTGCGACAG  
 GTAGCCACAGCT  
 ATATCTATTTCCTGTTATTGCTGCAGGAGGAATTGCGGATGGTGAAGGTGCTGCGGCTGGCTTTATGCT  
 10 AGGTGCAGAGGC  
 TGTACAGGTGGGGACACGGTTTGTAGTTGCAAAAGAGTCGAATGCCCATCCAACTACAAGGAGAAAA  
 TTTTAAAAGCAA  
 GGGATATTGATACTACGATTTTCAGCTCAGCACTTTGGTCATGCTGTTTCGTGCTATTAAAAATCAGTTG  
 ACTAGAGATTTT  
 15 GAACTGGCTGAAAAAGATGCCTTTAAGCAGGAAGATCCTGATTTAGAAATCTTTGAACAAATGGGAGC  
 AGGTGCCCTAGC  
 CAAAGCAGTTGTTACGGTGATGTGGATGGTGGCTCTGTCATGGCAGGTCAAATCGCAGGGCTTGTTT  
 CTAAAGAAGAAA  
 CAGCTGAAGAAATCCTAAAAGATTTGTATTACGGAGCCGCTAAGAAAATTCAAGAAGAAGCCTCTCGC  
 20 TGGGCAGGAGTT  
 GTAAGAAATGACTAA-3'

(B) *Streptococcus pneumoniae* FabK polypeptide sequence deduced from a polynucleotide sequence in this table [SEQ ID NO:2].

25 NH<sub>2</sub>-  
 MKTRITELLKIDYPIFQGGMAWVADGDLGAVSKAGGLGIIGGGNAPKEVVKANIDKIKSLTDKPFQVN  
 IMLSPFVEDIVDLVIEEGVKVVTGAGNPSKYMERFHEAGIIVIPVVPVSVALAKRMEKIGADAVIAEG  
 MEAGGHIGKLTMTLVRQVATAISIPVIAAGGIADGEGAAAGFMLGAEAVQVGTRFVVAKESNAHPNYK  
 EKILKARDIDTTISAQHFGHAVRAIKNQLTRDFELAEKDAFKQEDPDLEIFEQMGAGALAKAVVHGDVD  
 30 GGSVMAGQIAGLVSKEETAEEILKDLYYGAACKIQEEASRWAGVVRND.  
 -COOH

### 35 Deposited materials

A deposit comprising a *Streptococcus pneumoniae* 0100993 strain has been deposited with the National Collections of Industrial and Marine Bacteria Ltd. (herein "NCIMB"), 23 St. Machar Drive, Aberdeen AB2 1RY, Scotland on 11 April 1996 and assigned deposit number 40794. The deposit was described as *Streptococcus pneumoniae* 0100993 on deposit.

On 17 April 1996 a *Streptococcus pneumoniae* 0100993 DNA library in *E. coli* was similarly deposited with the NCIMB and assigned deposit number 40800. The *Streptococcus pneumoniae* strain deposit is referred to herein as "the deposited strain" or as "the DNA of the deposited strain."

5       The deposited strain comprises a full length FabK gene. The sequence of the polynucleotides comprised in the deposited strain, as well as the amino acid sequence of any polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein.

10       The deposit of the deposited strain has been made under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for Purposes of Patent Procedure. The deposited strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. The deposited strain is provided merely as convenience to those of skill in the art and is not an admission that a deposit is required for enablement, such as that required under 35 U.S.C. §112. A license may be required to make,  
15       use or sell the deposited strain, and compounds derived therefrom, and no such license is hereby granted.

20       In one aspect of the invention there is provided an isolated nucleic acid molecule encoding a mature polypeptide expressible by the *Streptococcus pneumoniae* 0100993 strain, which polypeptide is comprised in the deposited strain. Further provided by the invention are FabK polynucleotide sequences in the deposited strain, such as DNA and RNA, and amino acid sequences encoded thereby. Also provided by the invention are FabK polypeptide and polynucleotide sequences isolated from the deposited strain.

#### Polypeptides

25       FabK polypeptide of the invention is substantially phylogenetically related to other FabK polypeptides from other species.

30       In one aspect of the invention there are methods provided using polypeptides of *Streptococcus pneumoniae* referred to herein as "FabK" and "FabK polypeptides" as well as biologically, therapeutically or clinically useful variants thereof, and compositions comprising the same, useful in such methods.

      Among the particularly preferred embodiments of the invention are methods using variants of FabK polypeptide encoded by naturally occurring alleles of a FabK gene, particularly in candidate compound screening.



The present invention further provides methods using an isolated polypeptide that: (a) comprises or consists of an amino acid sequence that has at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:2 over the entire length of SEQ ID NO:2; (b) a polypeptide encoded by an isolated polynucleotide comprising or consisting of a polynucleotide sequence that has at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:1 over the entire length of SEQ ID NO:1; (c) a polypeptide encoded by an isolated polynucleotide comprising or consisting of a polynucleotide sequence encoding a polypeptide that has at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:2, over the entire length of SEQ ID NO:2.

The polypeptides of the methods of the invention include, for example, a polypeptide of Table 1 [SEQ ID NO:2] (in particular a mature polypeptide) as well as polypeptides and fragments, particularly those that has a biological activity of FabK, and also those that have at least 95% identity to a polypeptide of Table 1 [SEQ ID NO:2] and also include portions of such polypeptides with such portion of the polypeptide generally comprising at least 30 amino acids and more preferably at least 50 amino acids, particularly those portions possessing an activity of a wild type FabK.

The invention also includes methods using a polypeptide consisting of or comprising a polypeptide of the formula:



wherein, at the amino terminus, X is hydrogen, a metal or any other moiety described herein for modified polypeptides, and at the carboxyl terminus, Y is hydrogen, a metal or any other moiety described herein for modified polypeptides,  $R_1$  and  $R_3$  are any amino acid residue or modified amino acid residue,  $m$  is an integer between 1 and 1000 or zero,  $n$  is an integer between 1 and 1000 or zero, and  $R_2$  is an amino acid sequence of the invention, particularly an amino acid sequence selected from Table 1 or modified forms thereof. In the formula above,  $R_2$  is oriented so that its amino terminal amino acid residue is at the left, covalently bound to  $R_1$ , and its carboxy terminal amino acid residue is at the right, covalently bound to  $R_3$ . Any stretch of amino acid residues denoted by either  $R_1$  or  $R_3$ , where  $m$  and/or  $n$  is greater than 1, may be either a heteropolymer or a homopolymer, preferably a heteropolymer. Other preferred embodiments of the invention are provided where  $m$  is an integer between 1 and 50, 100 or 500, and  $n$  is an integer between 1 and 50, 100, or 500.

It is most preferred in the methods of the invention that the polypeptide used is derived from *Streptococcus pneumoniae*; however, it may also be obtained from other organisms of the

same taxonomic genus. Methods are also provided using polypeptides from organisms of the same taxonomic family or order, among other organisms as described elsewhere herein.

For the purposes of this invention, a fragment is a variant polypeptide having an amino acid sequence that is entirely the same as part but not all of any amino acid sequence of any polypeptide used in the methods of the invention. As with FabK polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region in a single larger polypeptide.

Preferred fragments used in the methods of the invention include, for example, truncation polypeptides having a portion of an amino acid sequence of Table 1 [SEQ ID NO:2], or of variants thereof, such as a continuous series of residues that includes an amino- and/or carboxyl-terminal amino acid sequence, particularly those possessing an enzymatic function of wild type FabK.

#### Antagonists and Agonists - Assays and Molecules

Polypeptides and polynucleotides described herein may be used to assess the binding or other effects of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See, *e.g.*, Coligan *et al.*, *Current Protocols in Immunology* 1(2): Chapter 5 (1991).

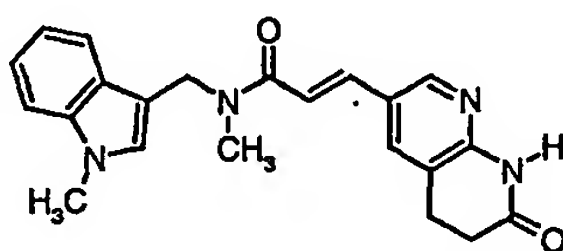
Polypeptides and polynucleotides described herein are responsible for many biological functions, including many disease states, in particular the Diseases herein mentioned, among others. In view of this, the present invention provides for a method of screening candidate compounds to identify those that agonize (*e.g.*, stimulate) or that antagonize (*e.g.*, inhibit) a function of a polypeptide or polynucleotide of the invention, as well as related polypeptides and polynucleotides. In general, agonists or antagonists may be employed for therapeutic and prophylactic purposes against such Diseases as herein mentioned. Compounds (herein also "candidate compound(s)") may be identified or selected from a variety of sources, for example, cells, cell-free preparations, known or newly synthesized compounds, chemical libraries, and natural product mixtures. Such compounds, such as agonists and antagonists, so-identified may be natural or modified substrates, ligands, receptors, enzymes, *etc.*, as the case may be, of FabK polypeptides and polynucleotides; or may be structural or functional mimetics thereof (see Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991)).

Antagonists of the invention include, among others, small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide and/or polypeptide of the invention

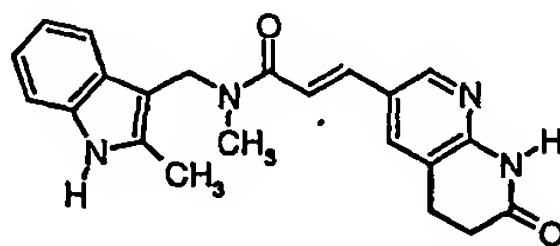


and thereby inhibit or extinguish its activity or expression. Antagonists also may be a small organic molecule, a peptide, a polypeptide, a closely related protein or antibody that binds the same sites on a binding molecule without inducing FabK-induced activities. Such antagonists preferably prevent the action or expression of FabK polypeptides and/or polynucleotides by  
 5 excluding FabK polypeptides and/or polynucleotides from binding.

Preferred embodiments of the invention comprise compounds of Formula I and Formula II, as well as homologues and stereoisomers thereof. These compounds are a representative set of compounds useful in the methods of the invention.



Formula I



Formula II

The compounds of Formula I and Formula II were prepared as set forth in the Examples.

Further preferred embodiments includes a composition comprising an antagonist or agonist of FabK polypeptide, particularly a small molecule, especially a small molecule having the structure of Formula I or Formula II, or is a homologue, stereoisomer, or a  
 25 pharmaceutically acceptable derivative thereof either compound.

FabK polypeptide selected from the group consisting of: (i) an isolated polypeptide comprising an amino acid having at least 95% identity to the amino acid sequence of SEQ ID NO:2 over the entire length of SEQ ID NO:2; (ii) an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2, (iii) an isolated polypeptide that is the amino acid  
 30 sequence of SEQ ID NO:2, and (iv) a polypeptide that is encoded by a recombinant polynucleotide comprising the polynucleotide sequence of SEQ ID NO:1, are also preferred.

Other antagonists or agonists of the invention include compounds that alter the binding of a cation to FabK, or alter an activity of a cation on FabK, particularly a monovalent cation. It is preferred that such antagonists inhibit such binding or lower such activity.

Preferred agonists and antagonists of the invention are those that also agonize or antagonize an activity of FabI. An agonist of FabI may act as an antagonist of FabK and *visa versa*, or such compounds may agonize or antagonize both FabI and FabK. The invention provides that these agonists and antagonists, when used as antimicrobial compounds, will be less likely to generate resistant mutants than for compounds that only act on a single target. This is a significant and surprising discovery in view of the teachings of Heath indicating that organisms that express FabK will be refractory to FabI inhibitors, and that bacteria possessing both targets will require a combination of inhibitors to block growth (Heath, *et al. Nature* 406: 145 (2000)). The compounds of Formulae I and II provide representative examples of compounds that antagonize both FabK and FabI.

Antagonists of the invention further include small molecules that bind to and occupy the binding site of a FabK polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules. Other antagonists include antisense molecules (see Okano, *J. Neurochem.* 56: 560 (1991); *OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION*, CRC Press, Boca Raton, FL (1988), for a description of these molecules).

Candidate compounds of the invention that are small molecules preferably have a molecular weight below 2,000 daltons, more preferably between 300 and 1,000 daltons, and most preferably between 400 and 700 daltons. It is particularly preferred that these small molecules are organic molecules.

25

#### Methods Using FabK Antagonists or Agonists

Any of the compounds or compositions of the invention may be used in methods of treatment provided herein. For example, the invention provides a method for treatment of bacterial infection comprising the step of contacting an individual with an antagonist or agonist of or a FabK polypeptide, particularly a FabK polypeptide selected from the group consisting of: (i) an isolated polypeptide comprising an amino acid having at least 95% identity to the amino acid sequence of SEQ ID NO:2 over the entire length of SEQ ID NO:2; (ii) an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2,

- (iii) an isolated polypeptide that is the amino acid sequence of SEQ ID NO:2, and
- (iv) a polypeptide that is encoded by a recombinant polynucleotide comprising the polynucleotide sequence of SEQ ID NO:1.

The compounds of the invention may also be used in a method for modulating the metabolism of bacteria comprising the step of contacting said bacteria with an antagonist or agonist of or a FabK polypeptide.

Further methods are provided wherein said antagonist is a small molecule.

Still further methods are provided wherein said small molecule has the structure or Formula I, or is a homologue, stereoisomer, or a pharmaceutically acceptable derivative thereof.

A method wherein said small molecule has the structure or Formula II, or is a homologue, or stereoisomer, or pharmaceutically acceptable derivative thereof is also provided by the invention.

A method is also provided wherein said infection is caused by or associated with a bacteria selected from the group consisting of: a member of the genus *Streptococcus*, *Staphylococcus*, *Bordetella*, *Corynebacterium*, *Mycobacterium*, *Neisseria*, *Haemophilus*, *Actinomycetes*, *Streptomycetes*, *Nocardia*, *Enterobacter*, *Yersinia*, *Francisella*, *Pasteurella*, *Moraxella*, *Acinetobacter*, *Erysipelothrix*, *Branhamella*, *Actinobacillus*, *Streptobacillus*, *Listeria*, *Calymmatobacterium*, *Brucella*, *Bacillus*, *Clostridium*, *Treponema*, *Escherichia*, *Salmonella*, *Klebsiella*, *Vibrio*, *Proteus*, *Erwinia*, *Borrelia*, *Leptospira*, *Spirillum*, *Campylobacter*, *Shigella*, *Legionella*, *Pseudomonas*, *Aeromonas*, *Rickettsia*, *Chlamydia*, *Borrelia* and *Mycoplasma*, and further including, but not limited to, a member of the species or group, Group A *Streptococcus*, Group B *Streptococcus*, Group C *Streptococcus*, Group D *Streptococcus*, Group G *Streptococcus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus faecalis*, *Streptococcus faecium*, *Streptococcus durans*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium diphtheriae*, *Gardnerella vaginalis*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium ulcerans*, *Mycobacterium leprae*, *Actinomycetes israelii*, *Listeria monocytogenes*, *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica*, *Escherichia coli*, *Shigella dysenteriae*, *Haemophilus influenzae*, *Haemophilus aegyptius*, *Haemophilus parainfluenzae*, *Haemophilus ducreyi*, *Bordetella*, *Salmonella typhi*, *Citrobacter freundii*, *Proteus mirabilis*, *Proteus vulgaris*, *Yersinia pestis*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Serratia liquefaciens*, *Vibrio cholera*, *Shigella dysenteriae*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Francisella tularensis*, *Brucella abortus*, *Bacillus anthracis*, *Bacillus*

*cereus, Clostridium perfringens, Clostridium tetani, Clostridium botulinum, Treponema pallidum, Rickettsia rickettsii, Helicobacter pylori* and *Chlamydia trachomatis*.

The contacting step in any of the methods of the invention may be performed in many ways that will be readily apparent to the skilled artisan. However, it is preferred that the contacting step is a provision of a composition comprising a FabK agonist or antagonist to a human patient in need of such composition or directly to bacteria in culture medium or buffer.

For example, when contacting a human patient or contacting said bacteria in a human patient or *in vitro*, the compositions comprising a FabK agonist or antagonist, preferably pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

#### Methods of Screening for FabK agonists and Antagonists

The screening methods of the invention may simply measure the binding of a candidate compound to a FabK polypeptide or polynucleotide, or to cells or membranes bearing a FabK polypeptide or polynucleotide, or a fusion protein of a FabK polypeptide by means of a label directly or indirectly associated with a candidate compound. Alternatively, the screening method may involve competition with a labeled competitor. Further, these screening methods may test whether a candidate compound results in a signal generated by activation or inhibition of a FabK polypeptide or polynucleotide, using detection systems appropriate to the cells comprising a FabK polypeptide or polynucleotide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Constitutively active polypeptide and/or constitutively expressed polypeptides and polynucleotides may be employed in screening methods for inverse agonists, in the absence of an agonist or antagonist, by testing whether the candidate compound results in inhibition of activation of the polypeptide or polynucleotide, as the case may be. Further, the screening methods may simply comprise the steps of mixing a candidate compound with a solution comprising a polypeptide or polynucleotide of the present invention, to form a mixture, measuring FabK polypeptide and/or polynucleotide activity in the mixture, and comparing the FabK polypeptide and/or polynucleotide activity of the mixture to a standard. Fusion proteins, such as those made from Fc portion and FabK polypeptide, as herein described, can also be used for high-throughput screening assays to identify antagonists of the polypeptide of the present invention, as well as of phylogenetically and and/or functionally related

polypeptides (see D. Bennett *et al.*, J Mol Recognition, 8:52-58 (1995); and K. Johanson *et al.*, J Biol Chem, 270(16):9459-9471 (1995)).

5 The polynucleotides, polypeptides and antibodies that bind to and/or interact with a polypeptide of the present invention may also be used to configure screening methods for detecting the effect of added compounds on the production of mRNA and/or polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents that may inhibit or enhance the production of polypeptide (also called antagonist or agonist, respectively) from suitably  
10 manipulated cells or tissues.

The invention also provides a method of screening candidate compounds to identify those that stimulate or inhibit an activity of FabK polypeptides or polynucleotides, particularly those compounds that are bacteriostatic and/or bactericidal. The method of screening may involve high-throughput techniques. For example, to screen for agonists or antagonists, a synthetic  
15 reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, comprising FabK polypeptide and a labeled substrate or ligand of such polypeptide is incubated in the absence or the presence of a candidate molecule that may be a FabK agonist or antagonist. The ability of a candidate molecule to agonize or antagonize the FabK polypeptide is reflected in decreased binding of the labeled ligand or decreased  
20 production of product from such substrate. Molecules that bind gratuitously, *i.e.*, without inducing the effects of FabK polypeptide are most likely to be good antagonists. Molecules that bind well and, as the case may be, increase the rate of product production from substrate, increase signal transduction, or increase chemical channel activity are agonists. Detection of the rate or level of, as the case may be, production of product from substrate, signal transduction, or  
25 chemical channel activity may be enhanced by using a reporter system. Reporter systems that may be useful in this regard include but are not limited to colorimetric, labeled substrate converted into product, a reporter gene that is responsive to changes in FabK polynucleotide or polypeptide activity, and binding assays known in the art.

Polypeptides of the invention may be used to identify membrane bound or soluble  
30 receptors, if any, for such polypeptide, through standard receptor binding techniques known in the art. These techniques include, but are not limited to, ligand binding and crosslinking assays in which the polypeptide is labeled with a radioactive isotope (for instance,  $^{125}\text{I}$ ), chemically modified (for instance, biotinylated), or fused to a peptide sequence suitable for detection or purification, and incubated with a source of the putative receptor (*e.g.*, cells,

cell membranes, cell supernatants, tissue extracts, bodily materials). Other methods include biophysical techniques such as surface plasmon resonance and spectroscopy. These screening methods may also be used to identify agonists and antagonists of the polypeptide that compete with the binding of the polypeptide to its receptor(s), if any. Standard  
5 methods for conducting such assays are well understood in the art.

The fluorescence polarization value for a fluorescently-tagged molecule depends on the rotational correlation time or tumbling rate. Protein complexes, such as formed by FabK polypeptide associating with a candidate compound, labeled to comprise a fluorescently-labeled molecule will have higher polarization values than a fluorescently  
10 labeled FabK protein not bound to a candidate compound. It is preferred that this method be used to characterize small molecules that bind FabK.

Surface plasmon resonance can be used to monitor the effect of small molecules on FabK polypeptide self-association as well as an association of FabK polypeptide and another polypeptide or small molecule. FabK polypeptide can be coupled to a sensor chip  
15 at low site density such that covalently bound molecules will be monomeric. Solution protein can then passed over the FabK polypeptide-coated surface and specific binding can be detected in real-time by monitoring the change in resonance angle caused by a change in local refractive index. This technique can be used to characterize the effect of small molecules on kinetic rates and equilibrium binding constants for FabK polypeptide self-  
20 association as well as an association of FabK polypeptide and another polypeptide or small molecule.

In other embodiments of the invention there are provided methods for identifying compounds that bind to or otherwise interact with and inhibit or activate an activity or expression of a FabK polypeptide and/or polynucleotide of the invention comprising: contacting  
25 a FabK polypeptide and/or polynucleotide of the invention with a compound to be screened under conditions to permit binding to or other interaction between the compound and the FabK polypeptide and/or polynucleotide to assess the binding to or other interaction with the compound, such binding or interaction preferably being associated with a second component capable of providing a detectable signal in response to the binding or interaction of the  
30 polypeptide and/or polynucleotide with the compound; and determining whether the compound binds to or otherwise interacts with and activates or inhibits an activity or expression of the FabK polypeptide and/or polynucleotide by detecting the presence or absence of a signal generated from the binding or interaction of the compound with the FabK polypeptide and/or polynucleotide.



Another example of an assay for FabK agonists or antagonists is a competitive assay that combines FabK and a potential agonist or antagonist with FabK-binding molecules, recombinant FabK binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. FabK can be labeled,  
5 such as by radioactivity or a colorimetric compound, such that the number of FabK molecules bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential agonist antagonist.

A further assay is provided whereby compounds that are agonists or antagonists of FabK are assayed to determine whether they are also agonists or antagonists of FabI, and *visa*  
10 *versa*. Embodiments are also provided where an assay mixture comprises both FabI and FabK.

FabK catalyses the reduction of enoyl-ACPs with the concomitant oxidation of NADH. Screening methods based on this reaction are preferred embodiments herein. Such methods may comprise the step of detecting reduction of crotonoyl-ACP to butyryl-ACP. Detection may be monitored, for example, by following the change in absorbance at a  
15 particular wavelength in the light spectrum, preferably at 340 nm, or by any other means of assaying NADH oxidation. Assays may be carried out, for example, in Costar 3696 half-area plates, preferably at a final assay volume of 150 ul on a Spectramax platereader. Preferred substrates used in the methods of the invention are NADH, NADPH, an NADH analogue, crotonoyl ACP and crotonyl CoA. Further provided are preferred methods  
20 comprising the step of incubating substrates with FabK enzyme in 100 mM N-[2-acetamido]-2 iminodiacetic acid (ADA), pH 6.5, 100 mM NH<sub>4</sub>Cl, 4% glycerol at 30°C, followed by an incubation step for 30 minutes after the addition of FabK. This reaction may be monitored at 340 nm, among other wavelengths.

A particularly preferred embodiment is a method comprising the step of providing  
25 monovalent cations to an assay mixture. This step enhances the activity of FabK enzyme. Any cation, or any compound that increases or enhances the activity of the enzyme, may be used in this method; however, NH<sub>4</sub><sup>+</sup> is preferred, and a concentration of 100 mM is most-preferred. Adding cations to the assay mixture is vastly superior to assaying in the absence of added cation. The activation in the reaction may be about 300-fold over the reaction  
30 with no monovalent cations added, such as in a cell-lysate assay without additional cations.

Using the assay methods of the invention, compounds may be tested for inhibition of FabK. It is preferred that the assay is performed using an assay volume of between 5 and 200 ul of candidate compound, more preferably 10 and 50 ul of candidate compound, and most preferably 30 ul. Preferred methods also utilize NADH, crotonoyl ACP and crotonyl

CoA in the assay mixture. In preferred embodiments of the methods of the invention, the final concentration in the assay mixture is between 10 uM and 50 uM crotonoyl ACP, but is most preferably 25 uM crotonoyl ACP; is between 20 uM and 100 uM NADH, but is most preferably 50 uM NADH; and is between 0.25 nM and 1.75 nM FabK enzyme, but is most preferably 1.25 nM FabK enzyme.

It is preferred that the assay mixtures are incubated at between 20 °C and 40 °C, preferably between 28 °C to 37 °C, and most preferably at about 30 °C. Preferred incubation times range between 30 seconds and 1 hour, are more preferably between 3 minutes and 30 minutes, and are most preferably about 4 or 5 minutes.

In certain preferred embodiments positive controls, without added candidate compound, are used in a reaction well to gauge the degree of agonism or antagonism of a candidate compound. Negative controls, without enzyme, may also be used.

#### **Compositions, kits and administration**

In a further aspect of the invention there are provided compositions comprising a FabK agonist or antagonist for administration to a cell or to a multicellular organism.

The invention also relates to compositions comprising an agonist or antagonist discussed herein or their agonists or antagonists. Agonists or antagonists of the invention may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to an individual. Such compositions comprise, for instance, a media additive or a therapeutically effective amount of an agonist or antagonist of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration. The invention further relates to diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Agonists or antagonists and other compounds of the invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds. In particular, FabK agonist or antagonists of the invention may be employed alone or in conjunction with other compounds, such as bacterial efflux pump inhibitor compounds or antibiotic compounds, particularly FabI agonists or antagonists.

The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

In therapy or as a prophylactic, the active agent may be administered to an individual  
5 as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

Alternatively the composition may be formulated for topical application  
for example in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops,  
mouthwash, impregnated dressings and sutures and aerosols, and may contain appropriate  
conventional additives, including, for example, preservatives, solvents to assist drug  
10 penetration, and emollients in ointments and creams. Such topical formulations may also  
contain compatible conventional carriers, for example cream or ointment bases, and ethanol  
or oleyl alcohol for lotions. Such carriers may constitute from about 1% to about 98% by  
weight of the formulation; more usually they will constitute up to about 80% by weight of the  
formulation.

15 In a further aspect, the present invention provides for pharmaceutical compositions  
comprising a therapeutically effective amount of a FabK agonist or antagonist, such as the  
soluble form of a compound of the present invention, an agonist or antagonist peptide or small  
molecule compound, in combination with a pharmaceutically acceptable carrier or excipient.  
Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol,  
20 ethanol, and combinations thereof. The invention further relates to pharmaceutical packs and  
kits comprising one or more containers filled with one or more of the ingredients of the  
aforementioned compositions of the invention.

The composition will be adapted to the route of administration, for instance by a  
systemic or an oral route. Preferred forms of systemic administration include injection,  
25 typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular,  
or intraperitoneal, can be used. Alternative means for systemic administration include  
transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids  
or other detergents. In addition, if a FabK agonist or antagonist can be formulated in an enteric  
or an encapsulated formulation, oral administration may also be possible. Administration of  
30 these compounds may also be topical and/or localized, in the form of salves, pastes, gels, and  
the like.

For administration to mammals, and particularly humans, it is expected that the daily  
dosage level of the active agent will be from 0.01 mg/kg to 10 mg/kg, typically around 1  
mg/kg. The physician in any event will determine the actual dosage that will be most suitable

for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

5           It is preferred that the dosage is selected to modulate metabolism of the bacteria in such a way as to inhibit or stop growth of said bacteria or by killing said bacteria. The skilled artisan may identify this amount as provided herein as well as by using other methods known in the art, *e.g.* by the application MIC tests.

10           In-dwelling devices include surgical implants, prosthetic devices and catheters, *i.e.*, devices that are introduced to the body of an individual and remain in position for an extended time. Such devices include, for example, artificial joints, heart valves, pacemakers, vascular grafts, vascular catheters, cerebrospinal fluid shunts, urinary catheters, continuous ambulatory peritoneal dialysis (CAPD) catheters.

15           The composition of the invention may be administered by injection to achieve a systemic effect against relevant bacteria shortly before insertion of an in-dwelling device. Treatment may be continued after surgery during the in-body time of the device. In addition, the composition could also be used to broaden perioperative cover for any surgical technique to prevent bacterial wound infections, especially wound infections from an organism described herein.

20           Many orthopedic surgeons consider that humans with prosthetic joints should be considered for antibiotic prophylaxis before dental treatment that could produce a bacteremia. Late deep infection is a serious complication sometimes leading to loss of the prosthetic joint and is accompanied by significant morbidity and mortality. It may therefore be possible to extend the use of the active agent as a replacement for prophylactic antibiotics in this  
25           situation.

          In addition to the therapy described above, the compositions of this invention may be used generally as a wound treatment agent to prevent adhesion of bacteria to matrix proteins exposed in wound tissue and for prophylactic use in dental treatment as an alternative to, or in conjunction with, antibiotic prophylaxis.

30           Alternatively, the composition of the invention may be used to bathe an indwelling device immediately before insertion. The active agent will preferably be present at a concentration of 1 $\mu$ g/ml to 10mg/ml for bathing of wounds or indwelling devices.

          A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit dose for vaccination is

0.5-5 microgram/kg of antigen, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks. With the indicated dose range, no adverse toxicological effects will be observed with the compounds of the invention that would preclude their administration to suitable individuals.

5

All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

10

## GLOSSARY

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

15

"Disease(s)" means any disease caused by or related to infection by an organism, particularly *Staphylococcus aureus* and *Streptococcus pneumoniae*, including, for example, otitis media, conjunctivitis, pneumonia, bacteremia, meningitis, sinusitis, pleural empyema and endocarditis, and most particularly meningitis, such as for example infection of cerebrospinal fluid.

20

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as the case may be, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Methods to determine identity are designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available computer

25

30

programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Altschul, S.F. et al., *J. Molec. Biol.* 215: 403-410 (1990)). The BLAST X program is publicly available from NCBI and other  
 5 sources (*BLAST Manual*, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., *J. Mol. Biol.* 215: 403-410 (1990)). The well known Smith Waterman algorithm may also be used to determine identity.

Parameters for polypeptide sequence comparison include the following: Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)  
 10 Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, *Proc. Natl. Acad. Sci. USA.* 89:10915-10919 (1992)  
 Gap Penalty: 12  
 Gap Length Penalty: 4

A program useful with these parameters is publicly available as the "gap" program from  
 15 Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

Parameters for polynucleotide comparison include the following: Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)  
 Comparison matrix: matches = +10, mismatch = 0  
 20 Gap Penalty: 50  
 Gap Length Penalty: 3  
 Available as: The "gap" program from Genetics Computer Group, Madison WI. These are the default parameters for nucleic acid comparisons.

A preferred meaning for "identity" for polynucleotides and polypeptides, as the case  
 25 may be, are provided in (1) and (2) below.

(1) Polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide sequence having at least a 95, 97 or 100% identity to the reference sequence of SEQ ID NO:1, wherein said polynucleotide sequence may be identical to the reference sequence of SEQ ID NO:1 or may include up to a certain integer number of  
 30 nucleotide alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or



in one or more contiguous groups within the reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

5

$$n_n \leq x_n - (x_n \cdot y),$$

wherein  $n_n$  is the number of nucleotide alterations,  $x_n$  is the total number of nucleotides in SEQ ID NO:1,  $y$  is 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and  $\cdot$  is the symbol for the multiplication operator, and wherein any non-integer product of  $x_n$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_n$ . Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

15

(2) Polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 95, 97 or 100% identity to a polypeptide reference sequence of SEQ ID NO:2, wherein said polypeptide sequence may be identical to the reference sequence of SEQ ID NO:2 or may include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of amino acid alterations is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

20

25

$$n_a \leq x_a - (x_a \cdot y),$$

30

wherein  $n_a$  is the number of amino acid alterations,  $x_a$  is the total number of amino acids in SEQ ID NO:2,  $y$  is 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and  $\cdot$  is the symbol for the multiplication operator, and wherein any non-integer product of  $x_a$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_a$ .

"Isolated" means altered "by the hand of man" from its natural state, *i.e.*, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. Moreover, a polynucleotide or polypeptide that is introduced into an organism by transformation, genetic manipulation or by any other recombinant method is "isolated" even if it is still present in said organism, which organism may be living or non-living.

"Organism(s)" means a (i) prokaryote, including but not limited to, a member of the genus *Streptococcus*, *Staphylococcus*, *Bordetella*, *Corynebacterium*, *Mycobacterium*, *Neisseria*, *Haemophilus*, *Actinomyces*, *Streptomyces*, *Nocardia*, *Enterobacter*, *Yersinia*, *Francisella*, *Pasturella*, *Moraxella*, *Acinetobacter*, *Erysipelothrix*, *Branhamella*, *Actinobacillus*, *Streptobacillus*, *Listeria*, *Calymmatobacterium*, *Brucella*, *Bacillus*, *Clostridium*, *Treponema*, *Escherichia*, *Salmonella*, *Klebsiella*, *Vibrio*, *Proteus*, *Erwinia*, *Borrelia*, *Leptospira*, *Spirillum*, *Campylobacter*, *Shigella*, *Legionella*, *Pseudomonas*, *Aeromonas*, *Rickettsia*, *Chlamydia*, *Borrelia* and *Mycoplasma*, and further including, but not limited to, a member of the species or group, Group A *Streptococcus*, Group B *Streptococcus*, Group C *Streptococcus*, Group D *Streptococcus*, Group G *Streptococcus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus faecalis*, *Streptococcus faecium*, *Streptococcus durans*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium diphtheriae*, *Gardnerella vaginalis*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium ulcerans*, *Mycobacterium leprae*, *Actinomyces israelii*, *Listeria monocytogenes*, *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica*, *Escherichia coli*, *Shigella dysenteriae*, *Haemophilus influenzae*, *Haemophilus aegyptius*, *Haemophilus parainfluenzae*, *Haemophilus ducreyi*, *Bordetella*, *Salmonella typhi*, *Citrobacter freundii*, *Proteus mirabilis*, *Proteus vulgaris*, *Yersinia pestis*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Serratia liquefaciens*, *Vibrio cholera*, *Shigella dysenteriae*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Francisella tularensis*, *Brucella abortus*, *Bacillus anthracis*, *Bacillus cereus*, *Clostridium perfringens*, *Clostridium tetani*, *Clostridium botulinum*, *Treponema pallidum*, *Rickettsia rickettsii*, *Helicobacter pylori* and *Chlamydia trachomatis*, (ii) an archaeon, including but not limited to *Archaeobacter*, and (iii) a unicellular or filamentous eukaryote, including but not limited to, a protozoan, a fungus, a member of the genus *Saccharomyces*, *Kluyveromyces*, or *Candida*, and a member of the species *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, or *Candida albicans*.

"Polynucleotide(s)" generally refers to any polyribonucleotide or polydeoxyribonucleotide, that may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotide(s)" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions or single-, double- and triple-stranded regions, 5 single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded, or triple-stranded regions, or a mixture of single- and double-stranded regions. In addition, "polynucleotide" as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the 10 same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. As used herein, the term "polynucleotide(s)" also includes DNAs or RNAs as described above that comprise one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other 15 reasons are "polynucleotide(s)" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term "polynucleotide(s)" as it is employed 20 herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including, for example, simple and complex cells. "Polynucleotide(s)" also embraces short polynucleotides often referred to as oligonucleotide(s).

"Polypeptide(s)" refers to any peptide or protein comprising two or more amino acids 25 joined to each other by peptide bonds or modified peptide bonds. "Polypeptide(s)" refers to both short chains, commonly referred to as peptides, oligopeptides and oligomers and to longer chains generally referred to as proteins. Polypeptides may comprise amino acids other than the 20 gene encoded amino acids. "Polypeptide(s)" include those modified either by natural processes, such as processing and other post-translational modifications, but also by chemical 30 modification techniques. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and they are well known to those of skill in the art. It will be appreciated that the same type of modification may be present in the same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may comprise many types of modifications. Modifications can occur anywhere in a

polypeptide, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl termini. Modifications include, for example, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins, such as arginylation, and ubiquitination. See, for instance, *PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES*, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993) and Wold, F., *Posttranslational Protein Modifications: Perspectives and Prospects*, pgs. 1-12 in *POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS*, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter et al., *Meth. Enzymol.* 182:626-646 (1990) and Rattan et al., *Protein Synthesis: Posttranslational Modifications and Aging*, Ann. N.Y. Acad. Sci. 663: 48-62 (1992). Polypeptides may be branched or cyclic, with or without branching. Cyclic, branched and branched circular polypeptides may result from post-translational natural processes and may be made by entirely synthetic methods, as well.

"Recombinant expression system(s)" refers to expression systems or portions thereof or polynucleotides of the invention introduced or transformed into a host cell or host cell lysate for the production of the polynucleotides and polypeptides of the invention.

"Variant(s)" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusion proteins and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more

substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. The present invention also includes include variants of each of the polypeptides of the invention, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acids are substituted, deleted, or added in any combination. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques, by direct synthesis, and by other recombinant methods known to skilled artisans.

#### EXAMPLES

The examples below are carried out using standard techniques, that are well known and routine to those of skill in the art, except where otherwise described in detail. The examples are illustrative, but do not limit the invention.

##### Example 1 Cloning of *Streptococcus pneumoniae* *FabK*

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The *S.pneumoniae* *fabK*, enoyl-ACP reductase gene was PCR amplified from *S.pneumoniae* strain 0100993. The forward and reverse primer sequences were 5' AGGTTGGAGGCCATATGAAAACGCGTATT 3' (SEQ ID NO:3) and 5' GGCGGATCCTTAGTCATTTCTTACAACCTC 3' (SEQ ID NO:4), respectively.

25 An *NdeI* site was integrated into the forward primer and a *BamHI* site into the reverse primer for cloning into pET24b(+). The PCR product was digested with the restriction endonucleases *NdeI* and *BamHI* and then ligated into pET24b(+), (also digested with *NdeI* and *BamHI*). The resulting plasmid was transformed into sub-cloning efficiency DH5-alpha cells. The sequence of the pET24bSpfabK expression construct was confirmed by DNA sequencing and the plasmid was transformed into electrocompetent BL21 (DE3) cells harboring the tRNA vector pRR692.

Intact FabK is expressed as 25% total cell protein of which 80% is soluble when induced with 0.1 mM IPTG at 37°C for three hours.



**Example 2 Purification of *S.pneumoniae* FabK**

One liter of cells containing the FabK expression construct were grown to an OD600 of 0.6. Expression was induced with 0.1 mM IPTG and the cells were grown for a further 3h and then harvested. The cell pellet was resuspended in 10 ml 50 mM Tris pH7.5, 1 mM PMSF, 1 mM Benzamidine, 1 mM DTT (buffer A) and lysed by sonication. Cell debris was removed by centrifugation. The supernatant was loaded onto a Hi-load Q (16/10) column (Pharmacia) equilibrated in buffer A. Protein was eluted over a 200 ml gradient of 0-100% buffer B, where buffer B is buffer A + 1 M KCl. Fractions containing FabK were identified by their absorbance at A460 and by their FabK activity and pooled.

1.5 M ammonium sulphate was added to the pooled fractions and these were then loaded onto a Hi-load Phenyl sepharose (16/10) column (Pharmacia) equilibrated in 50 mM Tris pH 7.5, 1 mM PMSF, 1 mM Benzamidine, 1 mM DTT, 1.5 M ammonium sulphate. Proteins were eluted with a gradient of ammonium sulphate (1.5 to 0 M) over 200 ml. Fractions containing FabK were identified as above and pooled. The pooled fractions were buffer exchanged into 100 mM Tris, pH 7.5, 2 mM DTT and glycerol was then added to 50%. The protein was stored at -20°C. It is preferred that the enzyme be stored with NH<sub>4</sub>Cl, which has been found to stabilize the enzyme.

The amino acid sequence of FabK in the Examples is:

MKTRITELLKIDYPIFQGGMAWVADGDLGAVSKAGGLGIIGGNAPKEVVKANID  
 KIKSLTDKPFQGVNIMLLSPFVEDIVDLVIEGVKVVTTGAGNPSKYMERFHEAGITVI  
 PVVPSVALAKRMEKIGADAVIAEGMEAGGHIGKLTMTLVRQVATAISIPVIAAGGI  
 ADGEGAAAGFMLGAEAVQVGTRFVVAKESNAHPNYKEKILKARDIDTTISAQHFG  
 HAVRAIKNQLTRDFELA EKDAFKQEDPDLEIFEQMGAGALAKAVVHGDVDGGSV  
 MAGQIAGLVSKEETAEEILKDL YYGAAKKIQEEASRWAGVVRND (SEQ ID NO:2)

**Example 3 FabK characterization**

The identity of the protein was confirmed by N-terminal sequencing and MALDI mass spectrometry. The optical spectrum of the protein was characteristic of flavoproteins, showing an absorbance in the 450nm region. The FAD cofactor was removed by denaturation of the protein and quantified. The ratio of FAD:protein was shown to be approximately 1:1.

**Example 4 Assaying the activity of FabK**



FabK catalyses the reduction of enoyl-ACPs with the concomitant oxidation of NADH. Crotonoyl-ACP can be prepared as described below. The reduction of crotonoyl-ACP to butyryl-ACP can be monitored by following the change in absorbance at 340 nm as NADH is oxidised.

5        Assays were carried out in Costar 3696 half-area plates in a final assay volume of 150  $\mu$ l on a Spectramax platereader. Substrates, NADH and crotonoyl ACP, were incubated with FabK enzyme in 100 mM N-[2-acetamido]-2 iminodiacetic acid (ADA), pH 6.5, 100 mM  $\text{NH}_4\text{Cl}$ , 4% glycerol at 30°C and the reaction monitored at 340 nm. This assaying can also be performed using crotonyl CoA, NADPH or an NADH analogue as a  
10        substrate.

#### Example 5 Activation by monovalent cations

FabK was found to be activated by monovalent cations. The greatest activation was found to be with  $\text{NH}_4^+$  at 100 mM, which activated the reaction about 300-fold over the  
15        reaction with no monovalent cations.

#### Example 6 Compound Screening

Using the above assay, compounds can be tested for inhibition of FabK. 30  $\mu$ l of a candidate compounds is added to a well of the plate. 30  $\mu$ l of a 250  $\mu$ M stock of NADH is  
20        then added to the well. 60  $\mu$ l of a 67.5  $\mu$ M stock of Crotonoyl ACP is added to the well. The plate is incubated at 30°C for 5 min. 30  $\mu$ l of a 6.25 nM stock of enzyme is then added to the well (also preincubated at 30°C) to initiate the reaction. The plate is then monitored at A340nm for 30 min at 30°C. Positive controls are reactions without compound. Negative controls are reactions without enzyme and without compound. Final  
25        concentrations in the assay mixture are 25  $\mu$ M crotonoyl ACP, 50  $\mu$ M NADH, 1.25 nM enzyme.

Two compounds were found to inhibit FabK using this assay, those of Formula I and Formula II. These compounds also inhibit FabI.

#### 30        Example 7 Synthesis of Crotonoyl-ACP

Crotonoyl -ACP was synthesised using *S. pneumoniae* ACP synthase to catalyse the addition of a crotonoyl group from crotonoyl CoA to *E.coli* apo-acyl carrier protein (ACP).

To a reaction vessel containing 500 mg (58  $\mu$ mol) of *E. coli* apo-ACP in 20 mM  
35        Bis-Tris, pH 6.8, 5 mM  $\text{MgCl}_2$ , was added 76 mg (81  $\mu$ moles) of crotonoyl-CoA and 5 mg

of *S. pneumoniae* ACP synthase. The final volume and pH were adjusted to 100 mL and 6.8, respectively. The pH of the reaction was maintained at 6.8 with NaOH and monitored for completion by mass spectrometry. Conversion was complete within 150 min with no detectable by-products. The reaction mixture was loaded at 10 mL/min onto a Q-Sepharose  
5 FF column (5 x 16 cm) pre-equilibrated with 20 mM Bis-Tris, pH 6.8. Crotonoyl-ACP was eluted over 2200 ml using a 0.2M-0.6M NaCl gradient at a flow rate of 20 ml/min. Fractions were monitored by mass spectrometry for identity and purity. The appropriate fractions were pooled and concentrated using a YM-3 membrane.

## 10 Example 8 FabI Assay Method

FabI enzyme, and methods of making and using it, is disclosed in patent applications numbered PCT/US00/12104 and EP1997000306506.

FabI catalyses the reduction of enoyl-ACPs with the concomitant oxidation of NAD(P)H. Crotonoyl-ACP can be prepared as described in patent applications numbered  
15 PCT/US00/12104 and EP1997000306506. The reduction of enoyl-ACPs can be monitored by following the change in absorbance at 340 nm as NADH is oxidised. Enoyl ACPs (eg crotonoyl-ACP) can be replaced by enoyl-CoAs (eg crotonoyl-CoA)

Assays were carried out in Costar 3696 half-area plates in a final assay volume of 150 ul on a Spectramax platereader. Substrates, NADH and crotonoyl ACP, were  
20 incubated with FabI enzyme in 100 mM N-[2-acetamido]-2 iminodiacetic acid (ADA), pH 6.5, 4% glycerol at 30°C and the reaction monitored at 340 nm. This assaying can also be performed using crotonyl CoA, NADPH or an NADH analogue as a substrate, or using a substrate suitable for FabK, such as those described above.

Using the above assay, compounds can be tested for inhibition of FabI. 30 ul of a  
25 candidate compounds is added to a well of the plate. 30 ul of a 250 uM stock of NADH is then added to the well. 60 ul of a 67.5 uM stock of Crotonoyl ACP is added to the well. The plate is incubated at 30°C for 5 min. 30 ul of a 6.25 nM stock of enzyme is then added to the well (also preincubated at 30°C) to initiate the reaction. The plate is then monitored at A340nm for 30 min at 30°C. Positive controls are reactions without compound.  
30 Negative controls are reactions without enzyme and without compound. Final concentrations in the assay mixture are 25 uM crotonoyl ACP and 50 uM NADH.

**Example 9 Synthesis of Formula I and Formula II and method of screening for antimicrobial activity**

SB No.	Structure	FabI IC <sub>50</sub> (uM)		Antibacterial MIC (ug/mL) <sup>a,b</sup>
		<i>S. aureus</i>	<i>E. coli</i>	
611113		0.10	<0.060	≤0.06 ( <i>S. aureus</i> Oxford) ≤0.06 ( <i>S. aureus</i> WCUH29) 16 ( <i>E. faecalis</i> I) 16 ( <i>E. faecalis</i> 7) 16 ( <i>H. influenzae</i> Q1) 32 ( <i>H. influenzae</i> NEMC1) ≤0.06 ( <i>M. catarrhalis</i> ) 8 ( <i>S. pneumoniae</i> 1629) 16 ( <i>S. pneumoniae</i> N1387) 8 ( <i>S. pneumoniae</i> ERY2) 8 ( <i>E. coli</i> AcrAB <sup>-</sup> )
641197				≤0.06 ( <i>S. aureus</i> Oxford) ≤0.06 ( <i>S. aureus</i> WCUH29) 16 ( <i>E. faecalis</i> I) 16 ( <i>E. faecalis</i> 7) 1 ( <i>H. influenzae</i> Q1) 2 ( <i>H. influenzae</i> NEMC1) ≤0.06 ( <i>M. catarrhalis</i> ) 8 ( <i>S. pneumoniae</i> 1629) 16 ( <i>S. pneumoniae</i> N1387) 8 ( <i>S. pneumoniae</i> ERY2) 0.5 ( <i>E. coli</i> AcrAB <sup>-</sup> )

- 5 In addition, two of our compounds have been found to be inhibitors of Streptococcus FabK: SB-611113 (IC<sub>50</sub> = 5.7 uM) and SB-641197 (IC<sub>50</sub> = 5.2 uM).

General

Proton nuclear magnetic resonance ( $^1\text{H}$  NMR) spectra were recorded at either 300 or 360 MHz, and chemical shifts are reported in parts per million ( $\delta$ ) downfield from the internal standard tetramethylsilane (TMS). Abbreviations for NMR data are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, app = apparent, br = broad. J indicates the NMR coupling constant measured in Hertz.  $\text{CDCl}_3$  is deuteriochloroform,  $\text{DMSO}-d_6$  is hexadeuteriodimethylsulfoxide, and  $\text{CD}_3\text{OD}$  is tetradeuteriomethanol. Mass spectra were obtained using electrospray (ES) ionization techniques. Elemental analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ. Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. All temperatures are reported in degrees Celsius. Analtech Silica Gel GF and E. Merck Silica Gel 60 F-254 thin layer plates were used for thin layer chromatography. Flash chromatography was carried out on E. Merck Kieselgel 60 (230-400 mesh) silica gel. Analytical HPLC was performed on Beckman chromatography systems. Preparative HPLC was performed using Gilson chromatography systems. ODS refers to an octadecylsilyl derivatized silica gel chromatographic support. YMC ODS-AQ® is an ODS chromatographic support and is a registered trademark of YMC Co. Ltd., Kyoto, Japan. PRP-1® is a polymeric (styrene-divinylbenzene) chromatographic support, and is a registered trademark of Hamilton Co., Reno, Nevada. Celite® is a filter aid composed of acid-washed diatomaceous silica, and is a registered trademark of Manville Corp., Denver, Colorado.

Preparation 1Preparation of 1-methyl-2-(methylaminomethyl)-1H-indole

## 5 a) Ethyl 1-methyl-1H-indole-2-carboxylate

NaH (60% dispersion in mineral oil, 8.02 g, 200.49 mmole) was washed with hexanes, then was suspended in dry DMF (530 mL). Solid ethyl indole-2-carboxylate (25.29 g, 133.66 mmole) was added portionwise over 5 - 10 min, allowing gas evolution to subside between additions. When the addition was complete, the yellow mixture was  
10 stirred for 15 min, then methyl iodide (42 mL, 668.3 mmole) was added all at once. The reaction was exothermic, and the internal temperature rose to 40 - 45°C. After 1 hr, the reaction was quenched with 10% NH<sub>4</sub>Cl (100 mL) and concentrated on the rotavap (high vacuum). The residue was partitioned between Et<sub>2</sub>O (500 mL) and H<sub>2</sub>O (100 mL), and the layers were separated. The Et<sub>2</sub>O layer was washed with H<sub>2</sub>O (100 mL), dried (MgSO<sub>4</sub>),  
15 and concentrated to leave the title compound (27.10 g, quantitative) as a light yellow solid. This was used without further purification: TLC (10% EtOAc/hexanes) R<sub>f</sub> = 0.39.

## b) N,1-Dimethyl-1H-indole-2-carboxamide

A suspension of ethyl 1-methyl-1H-indole-2-carboxylate (27.10 g, 133.34 mmole)  
20 in 40% aqueous CH<sub>3</sub>NH<sub>2</sub> (300 mL) and MeOH (30 mL) was stirred at RT. A solid tended to gradually creep up the walls of the flask, and was washed down periodically with MeOH. The flask was tightly stoppered to keep the material inside the flask. As the reaction proceeded, the solid dissolved, but eventually the product began to precipitate. The reaction was stirred at RT for 5 days, then was concentrated to remove approximately 200  
25 mL of the solvent. The remaining residue was diluted with H<sub>2</sub>O (300 mL), and the solid was collected by suction filtration and washed with H<sub>2</sub>O. Drying at 50 - 60°C in high vacuum left the title compound (23.45 g, 93%) as a faintly yellow solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.63 (d, J = 8.0 Hz, 1 H), 7.27 - 7.43 (m, 2 H), 7.10 - 7.20 (m, 1 H), 6.80 (s, 1 H), 6.10 - 6.30 (m, 1 H), 4.06 (s, 3 H), 3.01 (d, J = 4.9 Hz, 3 H).

30

## c) 1-Methyl-2-(methylaminomethyl)-1H-indole

A 3-liter 3-necked roundbottom flask equipped with overhead stirring was charged with N,1-dimethyl-1H-indole-2-carboxamide (23.45 g, 124.58 mmole) and anhydrous THF (170 mL). The solution was stirred while a solution of LiAlH<sub>4</sub> in THF (1.0 M, 250 mL,  
35 250 mmole) was added via syringe. Gas was evolved during the addition of the first 50 mL of LiAlH<sub>4</sub> solution. When the addition was complete, the resulting light yellow solution was heated at gentle reflux. After 23 hr, the reaction was cooled in ice and quenched by the

sequential dropwise addition of H<sub>2</sub>O (9.5 mL), 15% NaOH (9.5 mL), and H<sub>2</sub>O (28.5 mL). The mixture was stirred for 15 min, then was filtered through celite®, and the filter pad was washed thoroughly with THF. The filtrate was concentrated and the residue was flash chromatographed on silica gel (10% MeOH/CHCl<sub>3</sub> containing 0.5% conc. NH<sub>4</sub>OH). The  
5 title compound (20.17 g, 93%) was obtained as a light yellow oil: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.56 (d, J = 7.8 Hz, 1 H), 7.02 - 7.35 (m, 3 H), 6.38 (s, 1 H), 3.88 (s, 2 H), 3.75 (s, 3 H), 2.49 (s, 3 H).

### Preparation 2

10

#### Preparation of (E)-3-(6-aminopyridin-3-yl)acrylic acid

##### a) Benzyl (E)-3-(6-aminopyridin-3-yl)acrylate

A solution of 2-amino-5-bromopyridine (2.25 g, 13.0 mmole), benzyl acrylate (3.2  
15 g, 19.7 mmole), Pd(OAc)<sub>2</sub> (0.31 g, 1.4 mmole), tri-*ortho*-tolylphosphine (0.73 g, 2.4 mmole), and diisopropylethylamine (3.5 mL, 20.0 mmole) in propionitrile (50 mL) was heated at reflux overnight. The dark mixture was filtered through celite®, and the filtrate was concentrated. Flash chromatography on silica gel (3% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) gave the title compound (1.3 g, 39%): MS (ES) *m/e* 255 (M + H)<sup>+</sup>.

20

##### b) (E)-3-(6-Aminopyridin-3-yl)acrylic acid

A solution of benzyl (E)-3-(6-aminopyridin-3-yl)acrylate (1.3 g, 5.1 mmole) and 1.0 N NaOH (10 mL, 10 mmole) in MeOH was heated at reflux overnight. The solution was concentrated in vacuo, and the residue was dissolved in H<sub>2</sub>O. The pH was adjusted to  
25 6 with dilute HCl, and the solid precipitate was collected by suction filtration and dried to give the title compound (0.6 g, 72%) as a white solid: MS (ES) *m/e* 165 (M + H)<sup>+</sup>.



Preparation 3Preparation of (E)-3-[6-amino-5-(hydroxymethyl)pyridin-3-yl]acrylic acid

## 5 a) 2-Amino-3-(hydroxymethyl)pyridine

To a solution of 2-aminonicotinic acid (20.5 g, 148.1 mmole) in THF was added lithium aluminum hydride (300 mL, 1.0 M in THF) over 30 minutes. The reaction solution was heated to reflux for 18 hrs and then was cooled to room temperature. The reaction was quenched by the sequential dropwise addition of H<sub>2</sub>O (11.5 mL), 15% NaOH (11.5 mL),  
10 and H<sub>2</sub>O (34.5 mL). The mixture was stirred for 15 min, then was filtered through celite®, and the filter pad was washed thoroughly with THF followed by 5% CH<sub>3</sub>OH/CHCl<sub>3</sub>. The filtrate was concentrated to give the title compound (15.24 g, 83%) as a waxy light yellow solid: MS (ES) *m/e* 125 (M + H)<sup>+</sup>.

## 15 b) 2-Amino-5-bromo-3-(hydroxymethyl)pyridine

To a solution of 2-amino-3-(hydroxymethyl)pyridine (13.0 g, 116.0 mmole) in CH<sub>2</sub>Cl<sub>2</sub> (300 mL) at RT was added NBS (22.71 g, 127.6 mmole). After stirring at RT for 45 min the reaction solution was concentrated and the residue was dissolved in CHCl<sub>3</sub>. The resulting suspension was filtered and the filtrate was concentrated to a dark oil.  
20 Purification on silica gel (EtOAc) afforded the title compound (78%, 18.36 g) as a tan solid: MS (ES) *m/e* 204 (M + H)<sup>+</sup>.

## c) Benzyl (E)-3-[6-amino-5-(hydroxymethyl)pyridin-3-yl]acrylate

According to the procedure of Preparation 2 (a), except substituting 2-amino-3-(hydroxymethyl)-5-bromopyridine (1.10 g, 5.42 mmole) for 2-amino-5-bromopyridine, the  
25 title compound (1.25 g, 81%) was prepared as an off-white solid: MS (ES) *m/e* 285 (M + H)<sup>+</sup>.

## d) (E)-3-[6-Amino-5-(hydroxymethyl)pyridin-3-yl]acrylic acid

30 According to the procedure of Preparation 2 (b) except substituting benzyl-(E)-3-[6-amino-5-(hydroxymethyl)pyridin-3-yl]acrylate (1.10 g, 5.42 mmole) for benzyl (E)-3-(6-aminopyridin-3-yl)acrylate, the title compound (0.68 g, 65%) was prepared as an off-white solid: MS (ES) *m/e* 194 (M + H)<sup>+</sup>.

35

Preparation 4

Preparation of 4-methyl-5-(methylaminomethyl)-4H-thieno[3,2-b]pyrrole

a) Ethyl 4-methyl-4H-thieno[3,2-b]pyrrole-5-carboxylate

According to the procedure of Preparation 1 (a), except substituting ethyl 4H-thieno[3,2-b]pyrrole-5-carboxylate (1.30 g, 6.7 mmole, see *J. Het. Chem.* 1984, 21, 215-217) for ethyl indole-2-carboxylate, the title compound (1.35 g, 97%) was prepared as a yellow solid: MS (ES) *m/e* 210 (M + H)<sup>+</sup>.

b) N,4-Dimethyl-4H-thieno[3,2-b]pyrrole-5-carboxamide

According to the procedure of Preparation 1 (b), except substituting ethyl 4-methyl-4H-thieno[3,2-b]pyrrole-5-carboxylate (1.35 g, 6.5 mmole) for ethyl-1-methylindole-2-carboxylate, the title compound (1.19 g, 95%) was prepared as a yellow solid: MS (ES) *m/e* 195 (M + H)<sup>+</sup>.

c) 4-Methyl-5-(methylaminomethyl)-4H-thieno[3,2-b]pyrrole

According to the procedure of Preparation 1 (c), except substituting N,4-dimethyl-4H-thieno[3,2-b]pyrrole-5-carboxamide (0.70 g, 3.6 mmole) for N,1-dimethylindole-2-carboxamide, the title compound (0.60 g, 92%) was prepared as a yellow oil: MS (ES) *m/e* 181 (M + H)<sup>+</sup>.

The following examples illustrate methods for preparing the biologically active compounds of this invention from intermediate compounds such as those described in the foregoing Preparations.

Preparation of (E)-N-methyl-N-(3-methyl-1H-inden-2-ylmethyl)-3-(7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)acrylamide

a) N-Methyl-N-(3-methyl-1H-inden-2-ylmethyl)-acrylamide

To a solution of 3-methyl-2-(methylaminomethyl)indene hydrochloride (0.132 g, 0.63 mmole), from Preparation 4, and triethylamine (0.19 g, 1.89 mmole) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) at 0 °C was added a solution of acryloyl chloride (0.06 mL, 0.7 mmole) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL). The reaction was stirred at 0 °C for 1 hr, then was poured into water. The layers were separated, and the organic layer was washed with brine, dried over Mg SO<sub>4</sub> and concentrated in vacuo to yield the title compound (0.145 g, quantitative) as an oily solid: MS (ES) *m/e* 228.2 (M + H)<sup>+</sup>.

b) (E)-N-Methyl-N-(3-methyl-1*H*-inden-2-ylmethyl)-3-(7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)acrylamide

A mixture of 6-bromo-3,4-dihydro-1*H*-1,8-naphthyridin-2-one (0.096 g, 0.42 mmole), from Preparation 3, and N-methyl-N-(3-methyl-1*H*-inden-2-ylmethyl)acrylamide (0.141 g, 0.62 mmole) in propionitrile (10 mL) was treated with (*i*-Pr)<sub>2</sub>NEt (0.15 mL, 0.08 mmole), palladium acetate (0.014 g, 0.062 mmole), and (*o*-tolyl)<sub>3</sub>P (0.025 g, 0.08 mmole), and the resulting mixture was heated at gentle reflux. After 18 hr, the reaction was cooled, filtered through celite®, and concentrated. Flash chromatography on silica gel (2% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) gave the title compound (0.06 g, 41%) as a glassy solid: MS (ES) *m/e* 374.2 (M + H)<sup>+</sup>. Anal. Calcd for C<sub>23</sub>H<sub>23</sub>N<sub>3</sub>O<sub>2</sub> · 1.25 H<sub>2</sub>O: C, 69.76; H, 6.41; N, 10.61. Found: C, 69.86; H, 6.67; N, 10.51.

Preparation of (E)-N-methyl-N-(1-methyl-1*H*-indol-3-ylmethyl)-3-(7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)acrylamide

According to the procedure of Example 5, except substituting 1-methyl-3-(methylaminomethyl)indole (0.75 g, 3.3 mmole) for 3-methyl-2-(methylaminomethyl)indene hydrochloride, the title compound (0.59 g, 72%) was prepared as a light yellow solid: MS (ES) *m/e* 375 (M + H)<sup>+</sup>.

Preparation of (E)-N-methyl-N-(2-methyl-1*H*-indol-3-ylmethyl)-3-(7-oxo-5,6,7,8-tetrahydro-1,8-naphthyridin-3-yl)acrylamide

According to the procedure of Example 5, except substituting 2-methyl-3-(methylaminomethyl)indole (1.40 g, 8.00 mmole) for 3-methyl-2-(methylaminomethyl)indene hydrochloride, the title compound (1.30 g, 65%) was prepared as a light yellow solid: MS (ES) *m/e* 376 (M + H)<sup>+</sup>.

**What is claimed is:**

1. A method for the treatment of bacterial infection comprising the step of contacting an individual with an antagonist or agonist of a FabK polypeptide.

5

2. The method of claim 1 wherein said FabK polypeptide selected from the group consisting of:

- (i) an isolated polypeptide comprising an amino acid having at least 95% identity to the amino acid sequence of SEQ ID NO:2 over the entire length of SEQ ID NO:2;
- 10 (ii) an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2,
- (iii) an isolated polypeptide that is the amino acid sequence of SEQ ID NO:2, and
- (iv) a polypeptide that is encoded by a recombinant polynucleotide comprising the polynucleotide sequence of SEQ ID NO:1.

15

3. The method of claim 1 wherein said antagonist is a small molecule.

4. The method of claim 3 wherein said small molecule has the structure or Formula I, or a homologue, stereoisomer, or a pharmaceutically acceptable derivative thereof.

20

5. The method of claims 3 wherein said small molecule has the structure or Formula II, or a homologue, or stereoisomer, or pharmaceutically acceptable derivative thereof.

25

6. The method of claim 1 wherein said infection is caused by or associated with a bacteria selected from the group consisting of:

a member of the genus *Streptococcus*, *Staphylococcus*, *Bordetella*, *Corynebacterium*, *Mycobacterium*, *Neisseria*, *Haemophilus*, *Actinomycetes*, *Streptomyces*, *Nocardia*, *Enterobacter*, *Yersinia*, *Fancisella*, *Pasturella*, *Moraxella*, *Acinetobacter*, *Erysipelothrix*,  
30 *Branhamella*, *Actinobacillus*, *Streptobacillus*, *Listeria*, *Calymmatobacterium*, *Brucella*, *Bacillus*, *Clostridium*, *Treponema*, *Escherichia*, *Salmonella*, *Klebsiella*, *Vibrio*, *Proteus*, *Erwinia*, *Borrelia*, *Leptospira*, *Spirillum*, *Campylobacter*, *Shigella*, *Legionella*, *Pseudomonas*, *Aeromonas*, *Rickettsia*, *Chlamydia*, *Borrelia* and *Mycoplasma*, and further including, but not limited to, a member of the species or group, Group A *Streptococcus*, Group B *Streptococcus*,

Group C *Streptococcus*, Group D *Streptococcus*, Group G *Streptococcus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus faecalis*, *Streptococcus faecium*, *Streptococcus durans*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium diphtheriae*, *Gardnerella vaginalis*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium ulcerans*,  
 5 *Mycobacterium leprae*, *Actinomyces israelii*, *Listeria monocytogenes*, *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica*, *Escherichia coli*, *Shigella dysenteriae*, *Haemophilus influenzae*, *Haemophilus aegyptius*, *Haemophilus parainfluenzae*, *Haemophilus ducreyi*, *Bordetella*, *Salmonella typhi*, *Citrobacter freundii*, *Proteus mirabilis*, *Proteus vulgaris*,  
 10 *Yersinia pestis*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Serratia liquefaciens*, *Vibrio cholera*, *Shigella dysenteriae*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Franscisella tularensis*, *Brucella abortus*, *Bacillus anthracis*, *Bacillus cereus*, *Clostridium perfringens*, *Clostridium tetani*, *Clostridium botulinum*, *Treponema pallidum*, *Rickettsia rickettsii*, *Helicobacter pylori* and *Chlamydia trachomatis*.

15

7. A method for modulating the metabolism of bacteria comprising the step of contacting said bacteria with an antagonist or agonist of a FabK polypeptide.

8. The method of claim 7 wherein said FabK polypeptide selected from the group  
 20 consisting of:

- (i) an isolated polypeptide comprising an amino acid having at least 95% identity to the amino acid sequence of SEQ ID NO:2 over the entire length of SEQ ID NO:2;
- (ii) an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2,
- (iii) an isolated polypeptide that is the amino acid sequence of SEQ ID NO:2, and  
 25 (iv) a polypeptide that is encoded by a recombinant polynucleotide comprising the polynucleotide sequence of SEQ ID NO:1.

9. The method of claim 7 wherein said antagonist is a small molecule.

30 10. The method of claim 9 wherein said small molecule has the structure or Formula I, or a homologue, stereoisomer, or a pharmaceutically acceptable derivative thereof.

11. The method of claims 9 wherein said small molecule has the structure or Formula II, or a homologue, or stereoisomer, or pharmaceutically acceptable derivative thereof.

5 12. The method of claim 7 wherein said bacteria selected from the group consisting of:

a member of the genus *Streptococcus*, *Staphylococcus*, *Bordetella*, *Corynebacterium*, *Mycobacterium*, *Neisseria*, *Haemophilus*, *Actinomycetes*, *Streptomyces*, *Nocardia*, *Enterobacter*, *Yersinia*, *Fancisella*, *Pasturella*, *Moraxella*, *Acinetobacter*, *Erysipelothrix*,  
 10 *Branhamella*, *Actinobacillus*, *Streptobacillus*, *Listeria*, *Calymmatobacterium*, *Brucella*, *Bacillus*, *Clostridium*, *Treponema*, *Escherichia*, *Salmonella*, *Kleibsiella*, *Vibrio*, *Proteus*, *Erwinia*, *Borrelia*, *Leptospira*, *Spirillum*, *Campylobacter*, *Shigella*, *Legionella*, *Pseudomonas*, *Aeromonas*, *Rickettsia*, *Chlamydia*, *Borrelia* and *Mycoplasma*, and further including, but not limited to, a member of the species or group, Group A *Streptococcus*, Group B *Streptococcus*,  
 15 Group C *Streptococcus*, Group D *Streptococcus*, Group G *Streptococcus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus faecalis*, *Streptococcus faecium*, *Streptococcus durans*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium diphtheriae*, *Gardnerella vaginalis*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium ulcerans*,  
 20 *Mycobacterium leprae*, *Actinomycetes israelii*, *Listeria monocytogenes*, *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica*, *Escherichia coli*, *Shigella dysenteriae*, *Haemophilus influenzae*, *Haemophilus aegyptius*, *Haemophilus parainfluenzae*, *Haemophilus ducreyi*, *Bordetella*, *Salmonella typhi*, *Citrobacter freundii*, *Proteus mirabilis*, *Proteus vulgaris*, *Yersinia pestis*, *Kleibsiella pneumoniae*, *Serratia marcescens*, *Serratia liquefaciens*, *Vibrio*  
 25 *cholera*, *Shigella dysenterii*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Francisella tularensis*, *Brucella abortus*, *Bacillus anthracis*, *Bacillus cereus*, *Clostridium perfringens*, *Clostridium tetani*, *Clostridium botulinum*, *Treponema pallidum*, *Rickettsia rickettsii*, *Helicobacter pylori* and *Chlamydia trachomatis*.

30



## SEQUENCE LISTING

<110> SMITHKLINE BEECHAM CORPORATION  
SMITHKLINE BEECHAM p.l.c..

<120> METHODS OF AGONIZING AND ANTAGONIZING FABK

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